Transmission SIMS: A novel approach to achieving higher secondary ion yields of intact biomolecules

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There has been an increasing demand to extend accessible mass range in secondary ion mass spectrometry (SIMS) particularly for biological and biomedical molecular imaging. During the past two decades, various kinds of large cluster ions, such as C_{60} ions, argon gas cluster ions, water cluster ions, and metal cluster ions have been used as primary ions. It was shown that these cluster ions enhance emission of intact large molecular ions compared to monatomic ion bombardment. In SIMS, secondary ions emitted from a sample in the backward direction with respect to the incident direction of the primary ion are usually measured. If the sample is a self-supporting thin film, the secondary ions emitted in the forward direction upon transmission of the primary ions can be also measured. So far, there have been only few studies about the transmission SIMS. Boussofiane-Baudin *et al* found small enhancement of the secondary ion yield in the forward direction compared to the backward direction [1]. The origin of the enhancement was suggested to be the larger stopping power at the exit surface due to higher charge states achieved during the passage through the sample film. In this presentation, we demonstrate that large enhancement of the secondary ion yield of intact biomolecules can be achieved by combining the cluster ions (5 MeV C_{60}^+) with the transmission SIMS. We measured secondary ions emitted in the forward direction from phenylalanine amino acid films deposited on self-supporting amorphous Si_3N_4 (a-SiN) films. We found significant enhancement of the intact phenylalanine ion yield and large suppression of fragment ions compared to the backward direction [2].

Phenylalanine amino acid was purchased from Nakalai Tesque (Japan) and used without further purification. Self-supporting a-SiN films (1.5 × 1.5 mm^2) of thickness 20 – 50 nm were purchased from Silson Ltd (Northampton, UK). Thin films of phenylalanine (20 – 100 nm) were deposited on the a-SiN films using vacuum evaporation. The thickness and uniformity of the deposited phenylalanine films were examined by measuring energy loss spectra of 6 MeV Cu^{4+} ions passing through the phenylalanine/a-SiN films. A beam of 5 MeV C_{60}^+ ions was produced by a 3MV tandem accelerator at QST/Takasaki. The beam was collimated by an aperture (diameter 1mm) and sent to a scattering chamber (base pressure 1 × 10^{-6} Pa). For the SIMS measurements, the beam current was reduced to less than 0.1 fA. The collimated beam was incident on the phenylalanine/a-SiN film from the a-SiN side at 45° with respect to the surface normal. Mass spectra of secondary ions emitted in the forward direction were measured by a time-of-flight setup. We also measured the secondary ions emitted in the backward direction from the phenylalanine film using the same equipment.

Figure 1 shows the observed mass spectra of positive secondary ions emitted in the forward (solid line) and backward (dashed line) directions observed using 5 MeV C_{60}^+ ions. A peak of protonated intact phenylalanine ions $[Phe+H]^+$ is seen at m/z = 166. There are also many peaks corresponding to fragment ions, for example, $[Phe\text{-}COOH]^+$ ions at m/z = 120, $C_8H_8^+$ ions at m/z = 104, $C_7H_7^+$ ions at m/z = 91, $C_6H_5^+$ ions at m/z = 77, and so on. Both spectra

are similar but the yield of the intact phenylalanine ion is enhanced in the forward direction compared to the backward direction by a factor of 8. Similar enhancement is also seen for the large fragment ions, *e.g.* the yield of $[Phe\text{-}COOH]^+$ is enhanced by a factor of 4. For smaller fragment ions, however, the yield is reduced in the forward direction compared to the backward. The origin of these behaviors is attributed to a broader spatial distribution of constituent carbon ions at the exit surface due to the multiple scattering during the passage through the sample film. In the present case, FWHM of the distribution is estimated to be ~ 50 nm using the SRIM code, which is about 70 times larger than the diameter of C_{60}^+. As a result, the distribution of the deposited energy is broader with a lower peak energy density at the exit surface. Such a distribution is preferable for the soft ionization which enhance the yield of intact molecular ions and suppress fragmentation.

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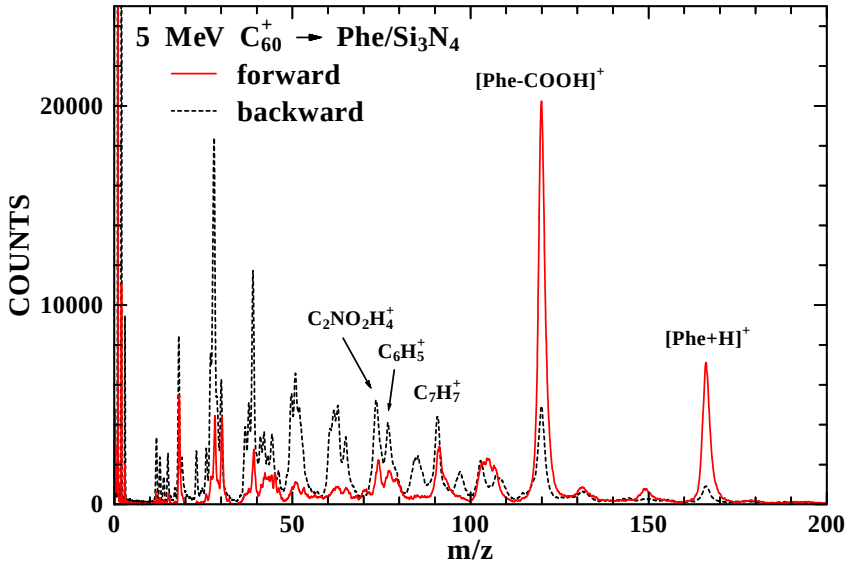

Figure 1. Mass spectra for positive secondary ions from phenylalanine/a-SiN films under 5 MeV C_{60}^+ ion bombardment. The spectra observed in the forward direction (solid line) and the backward direction (dashed line) are shown.

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